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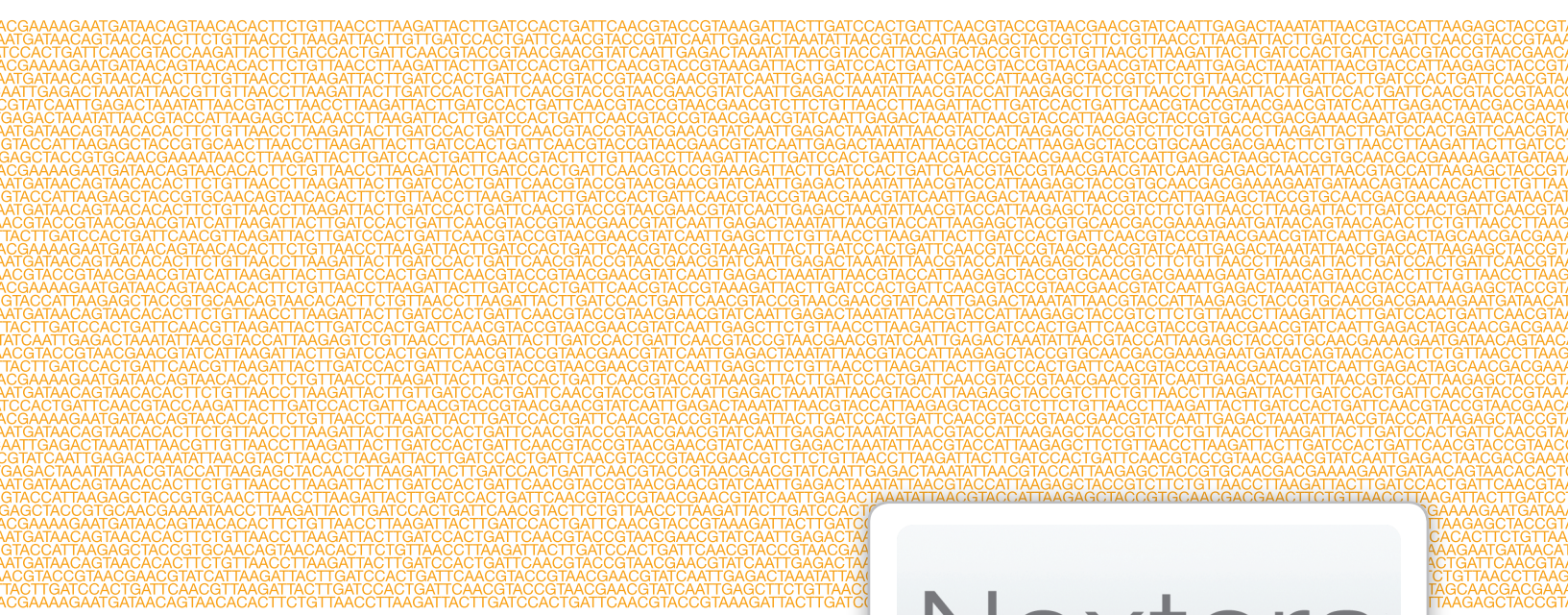
Nextera XT DNA Library Preparation Guide



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Revision History

Part #	Revision	Date	Description of Change
15031942	E	January 2015	• Corrected info for Nextera XT DNA Library Preparation Index Kit v2 Set A (FC-131-2001) to include index N715.
15031942	D	September 2014	• Added info for new index kits that enable preparation of up to 384 indexed paired-end libraries. • Updated *DNA Input Recommendations* for diluting starting material and the potential results of incomplete tagmentation. • Added new *Nextera XT Quality Metrics* on page 30 with new information on how to troubleshoot fluctuations in cluster density. • Removed Dual Indexing Principle and Low Plexity Pooling Guidelines sections. This information can be found in the Nextera Low-Plex Pooling Guidelines Tech Note on the Nextera XT DNA Library Preparation support page. • References to read lengths on the MiSeq were updated for v3 chemistry. • Added instructions for alternate tip if processing fewer than 24 samples while transferring LNB1 beads in *Library Normalization*. • Added NaOH 1N pH > 12.5 to the *Consumables and Equipment* list as a user-supplied consumable. • Removed Tween 20 from *Consumables and Equipment* list. Consumable not used in protocol.
15031942	C	October 2012	• Modifications were added in *PCR Clean-Up* for 2x300 runs on the MiSeq. • New section for clustering samples on the HiSeq, HiScanSQ, and GAIIx. See *Clustering Nextera XT Samples for HiSeq, HiScanSQ, and GAIIx* on page 24. • The *Dual Indexing Principle* section listed incorrect catalog numbers for the Nextera XT Index kits. The correct catalog numbers are now listed. • Added emphasis on making sure the NT (Neutralize Tagment Buffer) and LNS1 (Library Normalization Storage Buffer 1) reagents are at room temperature before use in the protocol. • Removed reference to *Tris-Cl 10mM, pH8.5 with 0.1% Tween 20* from the User-Supplied Consumables table because it is not used in this library preparation.
15031942	B	July 2012	• Added emphasis on making sure the NT (Neutralize Tagment Buffer) and LNS1 (Library Normalization Storage Buffer 1) reagents are at room temperature before use in the protocol. • Removed reference to Tris-Cl 10mM, pH8.5 with 0.1% Tween 20 from the User-Supplied Consumables table because it is not used in this library preparation.
15031942	A	May 2012	• Initial Release

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Overview

Chapter 1

Introduction

This protocol explains how to prepare up to 384 indexed paired-end libraries from various DNA for subsequent cluster generation and DNA sequencing. This protocol uses the reagents provided in the Illumina Nextera®XT DNA Library Preparation Kit. The goal of this protocol is to fragment and add adapter sequences onto template DNA with a single tube Nextera XT tagmentation reaction to generate multiplexed sequencing libraries.

The Nextera XT DNA Library Preparation protocol offers:

Sequencing's fastest and easiest preparation

- Single well enzymatic reaction both fragments and adds adapter in only 15 minutes, no mechanical fragmentation/shearing required
- Master mixed reagents to reduce reagent containers, pipetting and hands-on time
- Innovative sample normalization that eliminates the need for library quantification before sample pooling and sequencing

Lowest DNA input

- Only 1 ng input DNA needed

Single kit for many applications

- Easily prepare amplicons, small genomes, and plasmids
- Fastest method to prepare libraries for any Illumina sequencing system

Flexible throughput

- 384 indexes available and supported on all Illumina sequencing systems
- Master mixed reagents and automation-friendly configurations

Table 1 Example of Applications for Different Nextera Kits

Nextera (FC-131-1031)	Nextera XT (FC-131-1096)
Large / complex genomes	Small genomes, amplicons, plasmids
Human genomes	PCR Amplicons (> 300 bp)*
non-human mammalian genomes (e.g. mouse, rat, bovine)	Plasmids
Plant genomes (e.g. Arabidopsis, maize, rice)	Microbial Genomes (e.g. Prokaryotes, archaea)
Invertebrates genomes (e.g. Drosophila)	Concatenated Amplicons
	double-stranded cDNA

* Illumina recommends > 300 bp to ensure even coverage across the length of the DNA fragment. An expected drop off in sequencing coverage about 50 bp from each distal end of a fragment can be seen. The drop off is because the tagmentation reaction cannot add an adapter right at the distal end of a fragment. This enzymatic clipping of PCR primers avoids wasted sequencing output on non-informative bases that do not contain genomic inserts. If you wish to sequence the genomic loci contained within a PCR primer, simply design your amplicons to be ~100 bases larger than the desired insert to be sequenced.

DNA Input Recommendations

The Nextera XT DNA Library Preparation Kit protocol is optimized for 1 ng of input DNA total. Illumina strongly recommends quantifying the starting genomic material.

NOTE
Illumina recommends diluting starting material in molecular grade water or 10 mM Tris-HCl, pH 7.5–8.5.

Input DNA Quantitation

Nextera XT DNA Library Preparation library preps use an enzymatic DNA fragmentation step and thus can be more sensitive to DNA input compared to mechanical fragmentation methods. The ultimate success of the assay strongly depends on using an accurately quantified amount of input DNA library. Therefore, the correct quantitation of the DNA library is essential.

To obtain an accurate quantification of the DNA library, quantify the starting DNA library using a fluorometric based method. The method chosen must be for duplex DNA such as the Qubit dsDNA BR Assay system. Illumina recommends using 2 µl of each DNA sample with 198 µl of the Qubit working solution for sample quantification. Avoid methods that measure total nucleic acid content (e.g. nanodrop or other UV absorbance methods). Common contaminants such as ssDNA, RNA, and oligos are not substrates for the Nextera XT assay.

Assessing DNA Quality

UV absorbance is a commonly used method to assess the quality of a DNA sample. The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity. This protocol is optimized for DNA with absorbance ratio values of 1.8–2.0, which eliminates the presence of EDTA in your samples.

Place your starting DNA in either elution buffer or water. Incomplete tagmentation potentially leads to library preparation failure, poor clustering, or a higher than expected scaffold number.

Additional Resources

The following documentation is available for download from the Illumina website.

Resource	Description
Nextera XT DNA Library Preparation Experienced User Card and Lab Tracking Form (part # 15031943)	Provides protocol instructions, but with less detail than what is provided in this user guide. **New or less experienced users are advised to follow this user guide and not the EUC and LTF.**
Sample Sheet Guide and *IEM Nextera Quick Reference Card (part # 15037155)*	Provide information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and record parameters for your sample plate.
Analysis Software Guide	Provides information about the BaseSpace® sequencing data analysis tool that also enables you to organize samples, libraries, pools, and sequencing runs in a single environment.
Nextera XT DNA Library Preparation Low-Plex Pooling Guidelines Tech Note	Provides pooling guidelines and dual indexing strategies for Nextera XT DNA Library Preparation library preparation.

Visit the Nextera XT DNA Library Preparation support page on the Illumina website for access to additional documentation, software downloads, online training, frequently asked questions, and best practices.

Protocol

Chapter 2

Introduction

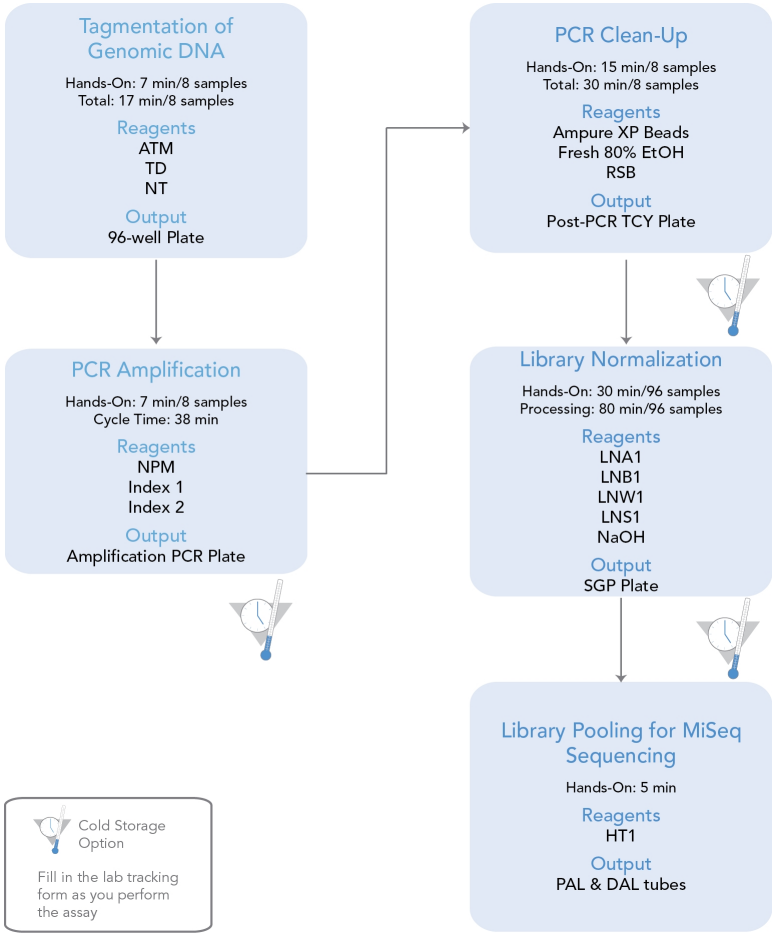
This chapter describes the Nextera XT DNA Library Preparation protocol.

- Review Best Practices before proceeding. See *Additional Resources* on page 4 for information on how to access Nextera XT DNA Library Preparation Best Practices on the Illumina website.
- Review Appendix A Supporting Information to confirm your kit contents and make sure that you have obtained all of the requisite equipment and consumables.
- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- If you are pooling, record information about your samples before beginning library preparation for later use in data analysis.
  - Do one of the following:
    - Use IEM to create and edit sample sheets for Illumina sequencing systems and analysis software. See *Additional Resources* on page 4 for information on how to download IEM software and documentation from the Illumina website.
    - Use BaseSpace to organize samples, libraries, pools, and a run for Illumina sequencing systems and analysis software. See *Additional Resources* on page 4 for information on how to access BaseSpace or download BaseSpace documentation from the Illumina website.
  - Include a common index in each column to facilitate pipetting operations when dispensing indexed adapters and pooling indexed libraries. See *Additional Resources* on page 4 for information on how to download the *Nextera Low-Plex Pooling Guidelines Tech Note*.

Nextera XT DNA Library Preparation Workflow

The following diagram illustrates the workflow using the Nextera XT DNA Library Preparation Kit. Safe stopping points are marked between steps.

Figure 1 Nextera XT DNA Library Preparation Workflow (For 8 samples)



Tagmentation of Input DNA

During this step, input DNA is tagmented (tagged and fragmented) by the Nextera XT transposome. The Nextera XT transposome simultaneously fragments the input DNA and adds adapter sequences to the ends, allowing amplification by PCR in subsequent steps.

Estimated Time (8 reactions)

- Hands-on: 7 minutes
- Total duration: 17 minutes

Consumables

Item	Quantity	Storage	Supplied By
ATM (Amplicon Tagment Mix)	1 tube	-25°C to -15°C	Illumina
TD (Tagment DNA Buffer)	1 tube	-25°C to -15°C	Illumina
NT (Neutralize Tagment Buffer)	1 tube	Room temperature	Illumina
Input DNA (0.2 ng/µl)		-25°C to -15°C	User
96-well hard shell TCY plate	1 plate		User
Microseal 'B' adhesive film			User

Preparation

1. Remove the ATM, TD, and input DNA from -25°C to -15°C storage and thaw on ice.
2. Visually inspect NT to make sure that there is no precipitate. If there is precipitate, vortex until all particulates are resuspended.
3. After thawing, mix reagents by gently inverting the tubes 3–5 times, followed by a brief spin in a microcentrifuge.

Make NTA

NOTE
Make sure that the reaction is assembled in the order described for optimal kit performance. You do not need to assemble the reaction on ice.

1. Label a new 96-well TCY plate **NTA** (Nextera XT Tagment Amplicon Plate).
2. Add 10 µl TD Buffer to each well to be used in this assay. Change tips between samples.

   NOTE
   Calculate the total volume of TD for all reactions, and divide the volume equally among the wells of a PCR eight-tube strip. Use a multichannel pipette to dispense into the **NTA** plate.

3. Add 5 µl input DNA at 0.2 ng/µl (1 ng total) to each sample well of the **NTA** plate.

   NOTE
   It is critical to use the full amount of input DNA.

4 Using a multichannel pipette, gently pipette up and down 5 times to mix. Change tips between samples.

5 Add 5 µl ATM to the wells containing input DNA and TD Buffer. Change tips between samples.

NOTE
Calculate the total volume of ATM for all reactions, and divide the volume equally among the wells of a PCR eight-tube strip. Use a multichannel pipette to dispense into the **NTA** plate.

6 Using a multichannel pipette, gently pipette up and down 5 times to mix. Change tips between samples.

7 Seal the **NTA** plate with a Microseal 'B' adhesive seal.

NOTE
For instructions on viewing a video demonstration of this process, see *Additional Resources* on page 4.

8 Centrifuge at 280 × g at 20°C for 1 minute.

9 Place the **NTA** plate in a thermal cycler and run the following program:

NOTE
Make sure that the thermal cycler lid is heated during the incubation.

- 55°C for 5 minutes
- Hold at 10°C

10 When the sample reaches 10°C, proceed immediately to *Neutralize NTA* as the transposome is still active.

Neutralize NTA

NOTE
Calculate the total volume of NT for all reactions, and divide the volume equally among the wells of a PCR eight-tube strip. Use a multichannel pipette to dispense into the **NTA** plate.

1 Carefully remove the Microseal 'B' seal and add 5 µl NT Buffer to each well of the **NTA** plate. Change tips between samples.

NOTE
For instructions on viewing a video demonstration of this process, see *Additional Resources* on page 4.

2 Using a multichannel pipette, gently pipette up and down 5 times to mix. Change tips between samples.

3 Seal the **NTA** plate with a Microseal 'B' adhesive seal.

4 Centrifuge at 280 × g at 20°C for 1 minute.

5 Place the **NTA** plate at room temperature for 5 minutes.

6 [Optional] Assess tagmentation by running 1 µl of sample on an HS bioanalyzer chip.

PCR Amplification

In this step, the tagmented DNA is amplified via a limited-cycle PCR program. The PCR step also adds index 1 (i7) and index 2 (i5) and sequences required for cluster formation. It is critical to use the full amount of recommended input DNA and not add extra cycles of PCR cycles to ensure libraries that produce high-quality sequencing results.

Estimated Time (8 reactions)

- Hands-on: 7 minutes
- Cycle time: 38 minutes
- Total duration: 45 minutes

Consumables

Item	Quantity	Storage	Supplied By
NPM (Nextera PCR Master Mix)	1 tube	-25°C to -15°C	Illumina
Index 1 primers (N7XX)	1 tube each index	-25°C to -15°C	Illumina
Index 2 primers (S5XX)	1 tube each index	-25°C to -15°C	Illumina
TruSeq Index Plate Fixture			Illumina
Microseal 'A' film			User

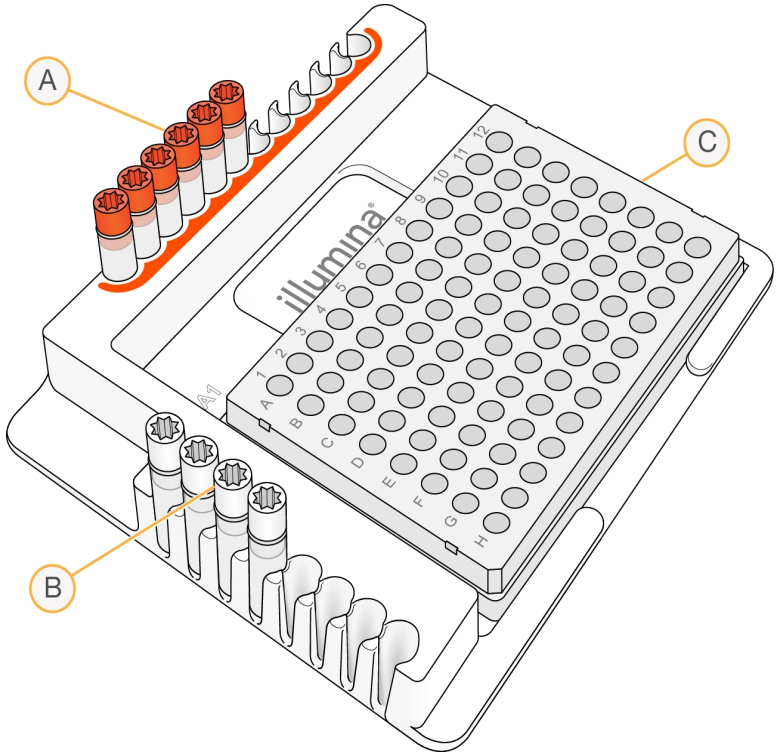
Preparation

1. If preparing the full set of 24/96 libraries for pooling and sequencing, proceed to step 2. If less than a full set of libraries is pooled for sequencing, make sure that the correct index 1 (i7) and index 2 (i5) primers have been selected. Use the Illumina Experiment Manager and the *Nextera XT DNA Library Preparation Low-Plex Pooling Guidelines Tech Note* to confirm the selected index primers.

   NOTE
   For instructions on viewing a video demonstration of this process, see *Additional Resources* on page 4.

2. Remove NPM and the index primers (i5 and i7) from -25°C to -15°C storage and thaw on a bench at room temperature.
   Allow approximately 20 minutes to thaw NPM and index primers.
3. After all reagents are thawed, gently invert each tube 3–5 times to mix and briefly centrifuge the tubes in a microcentrifuge. Use 1.7 ml Eppendorf tubes as adapters for the microcentrifuge.
4. For 24 libraries arrange the index primers in the TruSeq Index Plate Fixture using the following arrangement:
   a. Arrange index 1 (i7) primers (orange caps) in order horizontally.
   b. Arrange index 2 (i5) primers (white caps) in order vertically.
   c. To avoid index cross-contamination, discard the original caps and apply new caps provided in the kit.
   d. Record their positions on the Experienced User Card.

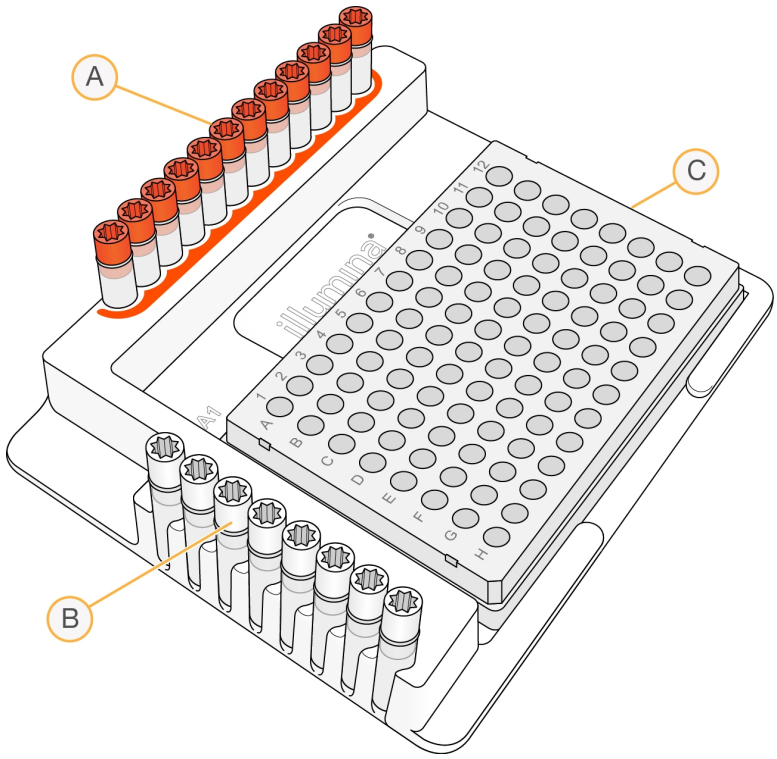
Figure 2 TruSeq Index Plate Fixture Arrangement (24 libraries)



A Index primer 1 (i7) (orange caps)
B Index primer 2 (i5) (white caps)
C NTA plate

5 For 96 libraries arrange the index primers in the TruSeq Index Plate Fixture using the following arrangement:
   a Arrange index 1 (i7) primer tubes (orange caps) in order horizontally.
   b Arrange index 2 (i5) primers (white caps) in order vertically.
   c To avoid index cross-contamination, discard the original caps and apply new caps provided in the kit.
   d Record their positions on the Experienced User Card.

Figure 3 TruSeq Index Plate Fixture (96 libraries)



A Index primer 1 (i7) (orange caps)
B Index primer 2 (i5) (white caps)
C NTA plate

Amplify NTA

1 Place the **NTA** plate in the TruSeq Index Plate Fixture.

2 Add 15 µl NPM to each well of the **NTA** plate containing index primers. Change tips between samples.

NOTE
Calculate the total volume of NPM for all reactions, and divide the volume equally among the wells of a PCR eight-tube strip. Use a multichannel pipette to dispense into the **NTA** plate.

3 Using a multichannel pipette, add 5 µl index 2 primers (white caps) to each column of the **NTA** plate. ***Changing tips between columns is required to avoid cross-contamination.***

4 Using a multichannel pipette, add 5 µl index 1 primers (orange caps) to each row of the **NTA** plate. ***Tips must be changed after each row to avoid index cross-contamination.***

5 To avoid index cross-contamination, discard the original *white* caps and apply new *white* caps provided in the kit.

6 To avoid index cross-contamination, discard the original *orange* caps and apply new *orange* caps provided in the kit. Remove all the index primer tubes from the working area.

7 Using a multichannel pipette, gently pipette up and down 5 times to mix. Change tips between samples to avoid index and sample cross-contamination.

8 Cover the **NTA** plate with Microseal 'A' film and seal with a rubber roller.

9 Centrifuge the **NTA** plate at 280 × g at 20°C for 1 minute.

10 Perform PCR using the following program on a thermal cycler:

NOTE
Make sure that the thermal cycler lid is heated during the incubation.

- 72°C for 3 minutes
- 95°C for 30 seconds
- 12 cycles of:
  - 95°C for 10 seconds
  - 55°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 10°C

SAFE STOPPING POINT
If you do not plan to proceed immediately to *PCR Clean-Up*, there are two options for storage. The **NTA** plate can remain on the thermal cycler overnight or you can store it at 2°C to 8°C for up to two days.

PCR Clean-Up

This step uses AMPure XP beads to purify the library DNA, and provides a size selection step that removes short library fragments from the population.

NOTE
For instructions on viewing a video demonstration of this process, see *Additional Resources* on page 4.

Estimated Time (8 reactions)

- Hands-on: 15 minutes
- Total duration: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
RSB (Resuspension Buffer)	1 tube	-25°C to -15°C	Illumina
AMPure XP beads		2°C to 8°C	User
Freshly prepared 80% ethanol (EtOH)			User
96-well MIDI plates	1 plate		User
96-well TCY plates	1 plate		User

Preparation

NOTE
Review the **Best Practices** section at the beginning of this protocol regarding the handling of magnetic beads and washing with 80% ethanol during the PCR clean-up.

1. Bring the AMPure XP beads to room temperature.
2. Prepare fresh 80% ethanol from absolute ethanol.

NOTE
Always prepare fresh 80% ethanol for wash steps. Ethanol can absorb water from the air impacting your results.

Make CAN

1. Centrifuge the **NTA** plate at 280 × g for 1 min (20˚C) to collect condensation.
2. Label a new MIDI plate **CAA** (Clean Amplified Plate).
3. Using a multichannel pipette set to 50 µl, transfer the PCR product from the **NTA** plate to the **CAA** plate. Change tips between samples.

NOTE
The ratio of PCR product to volume of beads is set at 3:2. (For example, 50 µl PCR product to 30 µl AMPure.) If you pull less than 50 µl of PCR product, adjust your ratio of AMPure beads accordingly.

4. Vortex the AMPure XP beads for 30 seconds to make sure that the beads are evenly dispersed. Add an appropriate volume of beads to a trough.

5 Using a multichannel pipette, add 30 µl AMPure XP beads to each well of the **CAA** plate.
For 2x250 or 2x300 runs on the MiSeq, add 25 µl of AMPure XP beads to each well of the **CAA** plate.
Smaller amplicon inputs into Nextera XT preps typically yield smaller insert size ranges. To maximize recovery of smaller fragments out of the SPRI cleanup, Illumina recommends the following conditions:

Size of Largest Amplicon in Pool	AMPure XP Recommendation	AMPure XP Volume
< 300 bp	1.8x AMPure XP*	90 µl
300–500 bp	1.8x AMPure XP	90 µl
> 500 bp	0.6x AMPure XP (0.5x AMPure XP for 2x250 runs on the MiSeq)	30 µl (25 µl for 2x250 or 2x300 runs on the MiSeq)

* Illumina recommends > 300 bp to ensure even coverage across the length of the DNA fragment. An expected drop off in sequencing coverage about 50 bp from each distal end of a fragment can be seen. The drop off is because the tagmentation reaction cannot add an adapter right at the distal end of a fragment. This enzymatic clipping of PCR primers avoids wasted sequencing output on non-informative bases that do not contain genomic inserts. If you wish to sequence the genomic loci contained within a PCR primer, simply design your amplicons to be ~100 bases larger than the desired insert to be sequenced.

6 Gently pipette mix up and down 10 times.

NOTE
Alternatively the solution can be mixed by shaking the **CAA** plate on a microplate shaker at 1800 rpm for 2 minutes.

7 Incubate at room temperature without shaking for 5 minutes.

8 Place the plate on a magnetic stand for 2 minutes or until the supernatant has cleared.

9 With the **CAA** plate on the magnetic stand, use a multichannel pipette to remove and discard the supernatant carefully. Change tips between samples.

NOTE
If any beads are inadvertently aspirated into the tips, dispense the beads back to the plate. Then let the plate rest on the magnet for 2 minutes and confirm that the supernatant has cleared.

10 With the **CAA** plate on the magnetic stand, wash the beads with freshly prepared 80% ethanol as follows:
   a Using a multichannel pipette, add 200 µl freshly prepared 80% ethanol to each sample well. Do not resuspend the beads yet.
   b Incubate the plate on the magnetic stand for 30 seconds.
   c Carefully remove and discard the supernatant.

11 With the **CAA** plate on the magnetic stand, perform a second ethanol wash as follows:
   a Using a multichannel pipette, add 200 µl freshly prepared 80% ethanol to each sample well.
   b Incubate the plate on the magnetic stand for 30 seconds.
   c Carefully remove and discard the supernatant.
   d Use a P20 multichannel pipette with fine pipette tips to remove excess ethanol.

12 With the **CAA** plate still on the magnetic stand, allow the beads to air-dry for 15 minutes.

13 Remove the **CAA** plate from the magnetic stand. Using a multichannel pipette, add 52.5 µl RSB to each well of the **CAA** plate.

14 Gently pipette mix up and down 10 times, changing tips after each column.

NOTE
Alternatively the solution can be mixed by shaking the **CAA** plate on a microplate shaker at 1800 rpm for 2 minutes.

15 Incubate at room temperature for 2 minutes.

16 Place the plate on the magnetic stand for 2 minutes or until the supernatant has cleared.

17 Label a new TCY plate **CAN** (Clean Amplified NTA Plate).

18 Using a multichannel pipette, carefully transfer 50 µl of the supernatant from the **CAA** plate to the **CAN** plate. Change tips between samples to avoid cross-contamination.

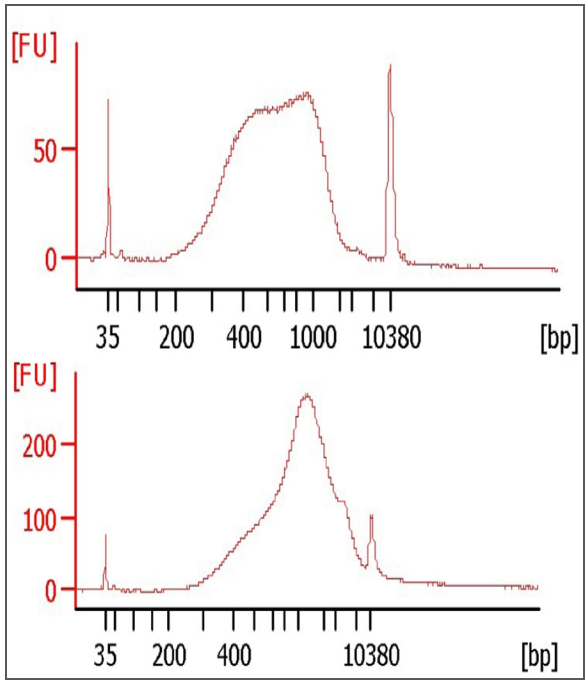
19 [Optional] See *Nextera XT Quality Metrics* on page 30 for information on how to troubleshoot fluctuations in cluster density.

SAFE STOPPING POINT
If you do not plan to proceed to *Library Normalization* immediately, seal the **CAN** plate with Microseal "B" adhesive seal and store it at -25°C to -15°C for up to a week.

Validate Library

It is optional to check the size distribution for some/all libraries by running 1 µl of undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip. Figure 4 shows example traces of successfully sequenced libraries. Typical libraries show a broad size distribution from ~250 bp to 1000 bp, as in the top panel. A wide variety of libraries can be sequenced, with average fragment sizes as small as 250 bp to as large as 1000–1500 bp.

Figure 4 Successful Human Genomic DNA Library Size Distributions Sequenced on HiSeq



Library Normalization

This process normalizes the quantity of each library to ensure more equal library representation in your pooled sample.

NOTE
For instructions on viewing a video demonstration of this process, see *Additional Resources* on page 4.

Estimated Time (96 reactions)

- Total duration: 1 hour 20 minutes
- Hands-on: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
LNA1 (Library Normalization Additives 1)	1 tube	-25°C to -15°C	Illumina
LNB1 (Library Normalization Beads 1)	1 tube	2°C to 8°C	Illumina
LNW1 (Library Normalization Wash 1)	2 tubes	2°C to 8°C	Illumina
LNS1 (Library Normalization Storage Buffer 1)	1 tube	Room temperature	Illumina
0.1 N NaOH (less than one week old)	3 ml per 96 samples		User
96-well MIDI plate	1 plate		User
96-well TCY plate	1 plate		User
15 ml conical tube	1 tube		User

WARNING
This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.
For more information, see the SDS for this kit, at support.illumina.com/sds.html.

Preparation

NOTE
Illumina recommends performing the LNA1 preparation step under a fume hood.

1 Remove LNA1 from -25°C to -15°C storage and bring to room temperature. Use a 20°C to 25°C water bath as needed.

NOTE
LNA1 might form visible precipitates or crystals. Before use, vortex vigorously, and then hold the tube in front of a light and visually inspect to make sure that all precipitate has dissolved.

2 Remove LNB1 and LNW1 from 2°C to 8°C storage and bring to room temperature. Use a 20°C to 25°C water bath as needed.

3 Vigorously vortex LNB1 for at least 1 minute with intermittent inversion. Repeat this step until the beads are well-resuspended and no pellet is found at the bottom of the tube when the tube is inverted.

4 Make sure that LNS1 is at room temperature before use.

Elute LNP

1 Label a new MIDI plate **LNP** (Library Normalization Plate).

2 Using a P20 multichannel pipette and fine tips, carefully transfer 20 µl of the supernatant from the **CAN** plate to the **LNP** plate. Change tips between samples to avoid cross-contamination.

3 For 96 samples, add 4.4 ml LNA1 to a fresh 15 ml conical tube.

4 Use a P1000 pipette set to 1000 µl to resuspend LNB1 thoroughly. Pipette up and down 15–20 times, until the bead pellet at the bottom is resuspended.

WARNING
It is critical to resuspend the LNB1 bead pellet at the bottom of the tube completely. The use of a P1000 ensures that the beads are homogeneously resuspended and that there is no bead mass at the bottom of the tube. This resuspension is essential for achieving consistent cluster density on the flow cell.

5 Immediately after LNB1 is thoroughly resuspended, use a P1000 pipette to transfer 800 µl LNB1 to the 15 ml conical tube containing LNA1. Mix well by inverting the tube 15–20 times. The resulting LNA1/LNB1 bead mix is enough for 96 samples. Pour the bead mix into a trough and use it immediately in the next step.

NOTE
If you do not plan to use full tubes for 96 samples, a P1000 set to 1000 µl is still required to resuspend the beads completely in step 4. Mix only the required amounts of LNA1 and LNB1 for the current experiment. Never use a P200 pipette to handle LNB1. Store the remaining LNA1 and LNB1 separately at their respective recommended temperatures. If not used immediately, never freeze or mix the LNB1 beads with LNA1 to preserve stability.

NOTE
If you are processing fewer than 24 samples, use a wide bore P200 tip or a P200 tip with the end cut off.

6 Using a multichannel pipette, add 45 µl combined LNA1/LNB1 to each well of the **LNP** plate containing libraries. If you use care to avoid cross-contamination, changing tips between columns is not required.

7 Seal the **LNP** plate with a Microseal 'B' adhesive seal.

8 Shake the **LNP** plate on a microplate shaker at 1800 rpm for 30 minutes.

NOTE
The 30 minute incubation is critical for proper library normalization. Incubations of greater or less than 30 minutes can affect library representation and cluster density.

9 Place the plate on a magnetic stand for 2 minutes and confirm that the supernatant has cleared.

10 With the **LNP** plate on the magnetic stand, use a multichannel pipette set to 80 µl to remove the supernatant and then discard in an appropriate hazardous waste container.

NOTE
If any beads are aspirated into the tips, dispense the beads back to the plate and let the plate rest on the magnet for 2 minutes, and then make sure that the supernatant is clear.

11 Remove the **LNP** plate from the magnetic stand and wash the beads with LNW1, as follows:

a Using a multichannel pipette, add 45 µl LNW1 to each sample well.
Changing tips is not required if you use care to avoid cross-contamination.
b Seal the **LNP** plate with a Microseal 'B' adhesive seal.
c Shake the **LNP** plate on a microplate shaker at 1800 rpm for 5 minutes.
d Place the plate on the magnetic stand for 2 minutes or until the supernatant has cleared.
e Carefully remove and discard the supernatant in an appropriate hazardous waste container.

12 Remove the **LNP** plate from the magnetic stand and repeat the wash with LNW1, as follows:

a Using a multichannel pipette, add 45 µl LNW1 to each well.
Changing tips between columns is not required if you use care to avoid cross-contamination.
b Seal the **LNP** plate with a Microseal 'B' adhesive seal.
c Shake the **LNP** plate on a microplate shaker at 1800 rpm for 5 minutes.
d Place the plate on the magnetic stand for 2 minutes or until the supernatant has cleared.
e Carefully remove and discard the supernatant in an appropriate hazardous waste container.

13 Remove the **LNP** plate from the magnetic stand and add 30 µl 0.1 N NaOH (fresh stock solution) to each well to elute the sample.

14 Seal the **LNP** plate with a Microseal 'B' adhesive seal.

15 Shake the **LNP** plate on a microplate shaker at 1800 rpm for 5 minutes.

16 During the 5 minute elution, apply the **SGP** (Storage Plate) barcode plate sticker to a new 96-well PCR plate.

17 Add 30 µl LNS1 to each well to be used in the **SGP** plate.

18 After the 5 minute elution, make sure that all samples in the **LNP** plate are resuspended completely. If the samples are not resuspended, gently pipette those samples up and down or lightly tap the plate on the bench to resuspend the beads. Then shake for another 5 minutes.

19 Place the **LNP** plate on the magnetic stand for 2 minutes or until the liquid is clear.

20 Using a multichannel pipette set to 30 µl, transfer the supernatant from the **LNP** plate to the **SGP** plate. Change tips between samples to avoid cross-contamination.

NOTE
If any beads are aspirated into the tips, dispense the beads back to the plate and let the plate rest on the magnet for 2 minutes, and then make sure that the supernatant is clear.

21 Seal the **SGP** plate with Microseal 'B' and then centrifuge to 1000 × g for 1 minute.

NOTE

Because the final library pool consists of single-stranded DNA, it does not resolve well on an agarose gel or Bioanalyzer chip. qPCR can be used for quality control if desired. For more information, please see the *Sequencing Library qPCR Quantification Guide (part # 11322363)*.

SAFE STOPPING POINT

If you do not plan to proceed to *Library Pooling and MiSeq Sample Loading* following the completion of Library Normalization, store the sealed **SGP** plate at -25°C to -15°C for up to one week.

Library Pooling and MiSeq Sample Loading

In preparation for cluster generation and sequencing, equal volumes of normalized library are combined, diluted in Hybridization Buffer, and heat denatured before sequencing.

Estimated Time (8 reactions)

- Total duration: 5 minutes
- Hands-on: 5 minutes

Estimated Time (24 reactions)

- Total duration: 10 minutes
- Hands-on: 10 minutes

Consumables

Item	Quantity	Storage	Supplied By
HT1 (Hybridization Buffer)	1 tube	-25°C to -15°C	Illumina
MiSeq reagent cartridge	1 cartridge	-25°C to -15°C	Illumina
Eppendorf LoBind Microcentrifuge Tube	1 tube		User
PCR 8-tube strip	1		User
2.5 L Ice bucket	1		User

Preparation

1. Set a heat block suitable for 1.5 ml centrifuge tubes to 96°C.
2. Remove a MiSeq reagent cartridge from -25°C to -15°C storage and thaw at room temperature.
3. In an ice bucket, prepare an ice-water bath by combining 3 parts ice and 1 part water.

Make DAL

NOTE
Prepare fresh **DAL** for each use.

1. If the **SGP** plate was stored frozen, thaw the plate at room temperature.
2. Centrifuge the **SGP** plate at 1000 × g for 1 minute at 20°C to collect condensation.
3. If the **SGP** plate was stored frozen, mix each library to be sequenced by pipetting up and down 3–5 times using a P200 multichannel pipette. Change tips between samples.
4. Using a P20 multichannel pipette, transfer 5 µl of each library to be sequenced from the **SGP** plate, column by column, to a PCR 8-tube strip. Change tips after each column to avoid cross-contamination.
5. Label a fresh Eppendorf tube **PAL** (Pooled Amplicon Library).

6 Combine and transfer the contents of the PCR 8-tube strip into the **PAL** tube. Mix PAL well.

7 Label a fresh Eppendorf tube **DAL** (Diluted Amplicon Library).

8 Add 576 µl HT1 to the **DAL** tube.

9 Transfer 24 µl **PAL** to the **DAL** tube containing HT1. Using the same tip, pipette up and down 3–5 times to rinse the tip and ensure complete transfer.

NOTE
This recommendation is for both MiSeq v2 and v3 reagents. The recommended volumes for diluting **PAL** with HT1 represent a 25-fold dilution. This dilution ratio was established by using the recommended equipment and following the normalization procedure strictly under typical laboratory conditions (e.g. 20°C to 25°C). An example of recommended equipment is a plate shaker that is calibrated for shaking speed. If cluster density is found to be too high or too low, change this dilution ratio to better suit the equipment, temperature, and user handling in your laboratory after validation.

10 Mix **DAL** by vortexing the tube at top speed.

11 Using a heat block, incubate the **DAL** tube at 96°C for 2 minutes.

12 After the incubation, invert **DAL** 1–2 times to mix and immediately place in the ice-water bath.

13 Keep the **DAL** tube in the ice-water bath for 5 minutes.

14 Load **DAL** into a thawed MiSeq reagent cartridge into the **Load Samples** reservoir.

NOTE
It is required to perform this heat denaturation step ***immediately*** before loading **DAL** into the MiSeq reagent cartridge to ensure efficient template loading on the MiSeq flow cell.

15 Cap the **PAL** tube and seal the **SGP** plate with a Microseal 'B' adhesive seal.

16 Store the **PAL** tube and the **SGP** plate at -25°C to -15°C for up to one week.

17 Sequence your library as indicated in the *MiSeq System User Guide (part # 15027617)*.

Clustering Nextera XT Samples for HiSeq, HiScanSQ, and GAIIx

In preparation for cluster generation and sequencing, equal volumes of normalized library are combined, diluted in Hybridization Buffer, and heat denatured prior to MiSeq sequencing. Libraries can be quantified after *PCR Clean-Up*. See *Nextera XT Quality Metrics* on page 30 for additional information. The steps in this section describe BBN for HiSeq, HiScanSQ, and GAIIx.

Estimated Time (8 reactions)

- Total duration: 5 minutes
- Hands-on: 5 minutes

Consumables

Item	Quantity	Storage	Supplied By
HT1 (Hybridization Buffer)	1 tube	-25°C to -15°C	Illumina
Eppendorf tubes (screw cap recommended)	2 tubes		User
PCR 8-tube strip	2		User

Make DAL

NOTE
Prepare fresh **DAL** for each use.

1. If the **SGP** plate was stored frozen, thaw the **SGP** plate at room temperature.
2. Centrifuge the **SGP** plate at 1,000 x g for 1 minute at 20°C to collect condensation.
3. If the **SGP** plate was stored frozen, mix each library to be sequenced by pipetting up and down 3–5 times using a P200 multichannel pipette. Change tips between samples.
4. Using a P20 multichannel pipette, transfer 5 µl of each library to be sequenced from the **SGP** plate, column by column, to a PCR 8-tube strip. Change tips after each column to avoid cross-contamination.
5. Label a fresh Eppendorf tube **PAL** (Pooled Amplicon Library).
6. Combine and transfer the contents of the PCR 8-tube strip into the PAL tube. Mix PAL well.
7. Label a fresh Eppendorf tube **DAL** (Diluted Amplicon Library).
8. Add 585 µl of HT1 to the **DAL** tube.
9. Transfer 15 µl of **PAL** to the **DAL** tube containing HT1. Using the same tip, pipette up and down 3–5 times to rinse the tip and ensure complete transfer.

NOTE

For both MiSeq v2 and v3 reagents, the recommended volumes for diluting **PAL** with HT1 represent a 40-fold dilution. This dilution ratio was established by using the recommended equipment (e.g. plate shaker calibrated for shaking speed) and following the normalization procedure strictly under typical laboratory conditions (e.g. 20°C–25°C). If cluster density is found to be too high or too low, change this dilution ratio. Change the dilution ratio to better suit the equipment, temperature, and user handling in your laboratory after validation.

10 Mix **DAL** by vortexing the tube at top speed.

11 Transfer 120 µl of the **DAL** tube into each well of the PCR 8-tube strip that is loaded onto the cBot for clustering.

NOTE
It is not required to perform heat denaturation before cBot loading because the clustering process includes a heat denaturation step.

12 Store the **PAL** tube and sealed **SGP** plate at -25°C to -15°C for up to a week.

13 Proceed to clustering and sequencing your library as indicated in the following guides:

- For all high output runs see the *cBot User Guide*.
- For HiSeq rapid runs, see the associated instrument guide.

Supporting Information

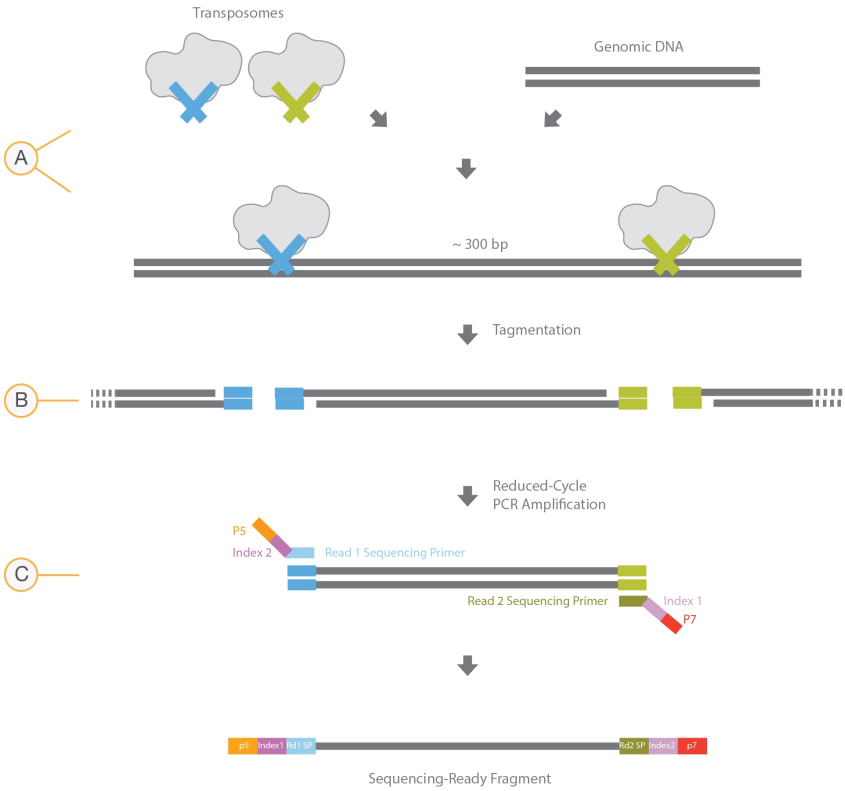
Appendix A

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all of the requisite consumables and equipment.

How does the Nextera XT Assay Work?

The Nextera XT DNA Library Preparation Kit uses an engineered transposome to simultaneously fragment and tag ("tagment") input DNA, adding unique adapter sequences in the process. A limited-cycle PCR reaction uses the adapter sequences to amplify the insert DNA. The PCR reaction also adds index sequences on both ends of the DNA, thus enabling dual-indexed sequencing of pooled libraries on any Illumina Sequencing System.



A Nextera XT transposome with adapters is combined with template DNA
B Tagmentation to fragment and add adapters
C Limited cycle PCR to add sequencing primer sequences and indexes

Nextera XT Quality Metrics

Two factors can cause cluster density fluctuations in libraries prepared with the Nextera XT DNA Library Preparation kit:

- The average sample size is too large or too small after tagmentation.
- The final sample concentration is too low due to a low yield when starting the bead-based normalization step.

To troubleshoot fluctuations in cluster density, consider checking library size and library concentration.

Check Library Size

Larger molecules cluster less efficiently than smaller molecules. If the fragment size after tagmentation is larger than expected, low cluster numbers are possible. The inverse is also true. The average expected library size after tagmentation is between 400 bp and 1.2 kb.

Illumina recommends that you check the library size with a high sensitivity bioanalyzer trace after the PCR cleanup step. Look for a long low plateau. Alternatively, PCR amplify the library with the KAPA QPCR primers and run the product on an agarose gel.

- **Short libraries indicate too little input DNA**—Illumina recommends requantifying the input DNA with a fluorometric method. If necessary, start with 10%–25% more input DNA. If the library peak is below 400 bp and you want to continue with this library, dilute the library further. Rather than 1:25, increase the final PAL dilution to 1:26 or 1:27 before sequencing.
- **Long libraries indicate too much input DNA or the presence of inhibitors**—Make sure that the input DNA is free from inhibitors, repeat quantification, and start with less input DNA. If you want to sequence a longer library, lower the final PAL dilution to 1:24 before sequencing.

Check Library Concentration

Bead-based normalization is most efficient when the library yield after PCR is 10–15 nM, or higher. Measure library concentration using HS dsDNA Qubit after PCR clean up, and measure library size with a Bioanalyzer to calculate molarity.

Typically, low library concentration after LNB normalization causes low input starting the normalization step. If you are starting with high-quality DNA and see low yield after PCR clean up, possible causes are issues with AMPure clean up or issues during PCR.

If your results show either condition, confirm proper storage of the PCR master mix at -25°C to -15°C in a no-frost freezer. Make sure that there were minimal freeze-thaw cycles.

For more information, Illumina recommends the following:

- **Best practices for bead handling**—From the Nextera XT DNA Library Preparation kit support page, click Best Practices tab and review Handling Magnetic Beads.
- **Online training module**—Review section 2.4 of the *TruSeq: Sample Purification Bead Size Selection and Best Practices*, which is a short training with guidance on bead handling. To access this training, click the Training tab on the Nextera XT DNA Library Preparation kit support page.

After normalization, you can expect constant final library concentration. However, certain issues can affect cluster density. To ensure quality results, check the library concentration after library normalization using high sensitivity ssDNA Qubit. Keep in mind that the library is single stranded for this calculation. Understanding library concentration after

library normalization helps in determining the appropriate final PAL dilution before sequencing.

Acronyms

Table 2 Nextera XT DNA Library Preparation Acronyms

Acronym	Definition
ATM	Amplicon Tagment Mix
CAA	Clean Amplified Plate
CAN	Clean Amplified NTA Plate
DAL	Diluted Amplicon Libraries
HT1	Hybridization Buffer
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNS1	Library Normalization Storage Buffer 1
LNW1	Library Normalization Wash 1
LNP	Library Normalization Plate
NT	Neutralize Tagment Buffer
NPM	Nextera PCR Master Mix
NTA	Nextera XT Tagment Amplicon Plate
PAL	Pooled Amplicon Library
RSB	Resuspension Buffer
SGP	Storage Plate
TD	Tagment DNA Buffer

Nextera XT DNA Library Preparation Kit

The Nextera XT DNA Library Preparation Kit is packaged in 96 or 24 sample boxes and shipped on dry ice unless specified otherwise. Each kit has a corresponding Index Kit that contains 96 or 24 indexes. Illumina offers multiple 96 index kits that allow up to 384 index kit combinations.

NOTE

Certain components of the kit are stored at a different temperature than the temperature at which they are shipped. As soon as you receive your kit, store the kit components at the specified temperature.

CAUTION

If sequencing Nextera XT libraries with HiSeq 2500/2000/1500/1000, HiScanSQ, or GAIIx, you must be sure to use the TruSeq Dual Index Sequencing Primer Boxes (Single Read or Paired End, as appropriate) for all sequencing run types: non-indexed, single-indexed, and dual-indexed. These sequencing primers are included for the MiSeq, NextSeq, and HiSeq rapid run mode. **If sequencing a Nextera XT library with the MiSeq System these add-on kits are not required.**

96 Samples

Consumable	Catalog #
Nextera XT DNA Library Preparation Kit	FC-131-1096
Nextera XT DNA Library Preparation Index Kit (96 Indexes, 384 Samples)	FC-131-1002
Nextera XT DNA Library Preparation Index Kit v2 Set A (96 Indexes, 384 samples)	FC-131-2001
Nextera XT DNA Library Preparation Index Kit v2 Set B (96 Indexes, 384 samples)	FC-131-2002
Nextera XT DNA Library Preparation Index Kit v2 Set C (96 Indexes, 384 samples)	FC-131-2003
Nextera XT DNA Library Preparation Index Kit v2 Set D (96 Indexes, 384 samples)	FC-131-2004

24 Samples

Consumable	Catalog #
Nextera XT DNA Library Preparation Kit	FC-131-1024
Nextera XT DNA Library Preparation Index Kit (24 Indexes, 96 Samples)	FC-131-1001

TruSeq Index Plate Fixture Kit

Illumina recommends using the index plate fixture to help with correctly arranging the index primers during the PCR Amplification steps. Each kit contains two fixtures and can be used for both the 24-sample kit and 96-sample kit.

Consumable	Catalog #
TruSeq Index Plate Fixture Kit	FC-130-1005

96 Sample Kit Contents (FC-131-1096)

Nextera XT DNA Library Preparation Kit

▸ Box 1

Quantity	Acronym	Reagent Name	Storage Temperature
1	ATM	Amplicon Tagment Mix, 96 RXN	-25°C to -15°C
2	TD	Tagment DNA Buffer	-25°C to -15°C
1	NPM	Nextera PCR Master Mix	-25°C to -15°C
4	RSB	Resuspension Buffer	-25°C to -15°C
1	LNA1	Library Normalization Additives 1	-25°C to -15°C
2	LNW1	Library Normalization Wash 1	2°C to 8°C
1	HT1	Hybridization Buffer	-25°C to -15°C

▸ Box 2

Quantity	Acronym	Reagent Name	Storage Temperature
1	NT	Neutralize Tagment Buffer	Room temperature
1	LNB1	Library Normalization Beads 1	2°C to 8°C
1	LNS1	Library Normalization Storage Buffer 1	Room temperature

Nextera XT DNA Library Preparation Index Kit (96 Indexes, 384 Samples) (FC-131-1002)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S502 to S508, and S517	-25°C to -15°C
12 tubes	Index Primers, N701 to N712	-25°C to -15°C

24 Sample Kit Contents (FC-131-1024)

Nextera XT DNA Library Preparation Kit

▸ Box 1

Quantity	Acronym	Reagent Name	Storage Temperature
1	ATM	Amplicon Tagment Mix, 24 RXN	-25°C to -15°C
1	TD	Tagment DNA Buffer	-25°C to -15°C
1	NPM	Nextera PCR Master Mix	-25°C to -15°C
1	RSB	Resuspension Buffer	-25°C to -15°C
1	LNA1	Library Normalization Additives 1	-25°C to -15°C
1	LNW1	Library Normalization Wash 1	2°C to 8°C
1	HT1	Hybridization Buffer	-25°C to -15°C

▸ Box 2

Quantity	Acronym	Reagent Name	Storage Temperature
1	NT	Neutralize Tagment Buffer	Room temperature
1	LNB1	Library Normalization Beads 1	2°C to 8°C
1	LNS1	Library Normalization Storage Buffer 1	Room temperature

Nextera XT DNA Library Preparation Index Kit (24 Indexes, 96 Samples)(FC-131-1001)

Quantity	Reagent Name	Storage Temperature
4 tubes	Index Primers, S502 to S504, and S517	-25°C to -15°C
6 tubes	Index Primers, N701 to N706	-25°C to -15°C

Nextera XT DNA Library Preparation Index Kit v2 Set A (FC-131-2001)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S502, S503, S505 to S508, S510, and S511	-25°C to -15°C
12 tubes	Index Primers, N701 to N707, N710 to N712, N714, and N715	-25°C to -15°C

Nextera XT DNA Library Preparation Index Kit v2 Set B (FC-131-2002)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S502, S503, S505 to S508, S510, and S511	-25°C to -15°C
12 tubes	Index Primers, N716, N718 to N724, and N726 to N729	-25°C to -15°C

Nextera XT DNA Library Preparation Index Kit v2 Set C (FC-131-2003)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S513, S515 to S518, and S520 to S522	-25°C to -15°C
12 tubes	Index Primers, N701 to N707, N710 to N712, N714, and N715	-25°C to -15°C

Nextera XT DNA Library Preparation Index Kit v2 Set D (FC-131-2004)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S513, S515 to S518, and S520 to S522	-25°C to -15°C
12 tubes	Index Primers, N716, N718 to N724, and N726 to N729	-25°C to -15°C

Consumables and Equipment

Check to make sure that you have all of the necessary user-supplied consumables and equipment before proceeding to library preparation. These consumables and equipment are Illumina recommended for the Nextera XT DNA Library Preparation protocols.

Table 3 User-Supplied Consumables

Consumable	Supplier
10 µl pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier
10 µl single channel pipettes	General lab supplier
1000 µl pipette tips	General lab supplier
1000 µl multichannel pipettes	General lab supplier
1000 µl single channel pipettes	General lab supplier
200 µl pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
96-well storage plates, round well, 0.8 ml ("MIDI" plate)	Fisher Scientific, part # AB-0859
Agencourt AMPure XP 60 ml kit	Beckman Coulter Genomics, part # A63881
Distilled water	General lab supplier
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
NaOH 1N pH > 12.5	General lab supplier
PCR grade water (for gel-free method)	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
Ultra pure water	General lab supplier
Microseal 96-well PCR plates ("TCY" plate)	Bio-Rad, part # HSP-9601

Table 4 User-Supplied Equipment

Equipment	Supplier
96-well thermal cycler (with heated lid)	See table in *Thermal Cycler* section.
Heat Block for 1.5 ml centrifuge tubes	General lab supplier
High-Speed microplate shaker	VWR, catalog # 13500-890 (110 V/120 V) VWR, catalog # 14216-214 (230 V)
Magnetic stand-96	Ambion, part # AM10027
Microplate centrifuge	General lab supplier
Vortexer	General lab supplier

Thermal Cycler

The following table lists the recommended settings for selected thermal cycler models. If your lab has not yet performed the Nextera XT DNA Library Preparation protocol, Illumina recommends that you validate any thermal cyclers not listed.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 5 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 6 Illumina Customer Support Telephone Numbers

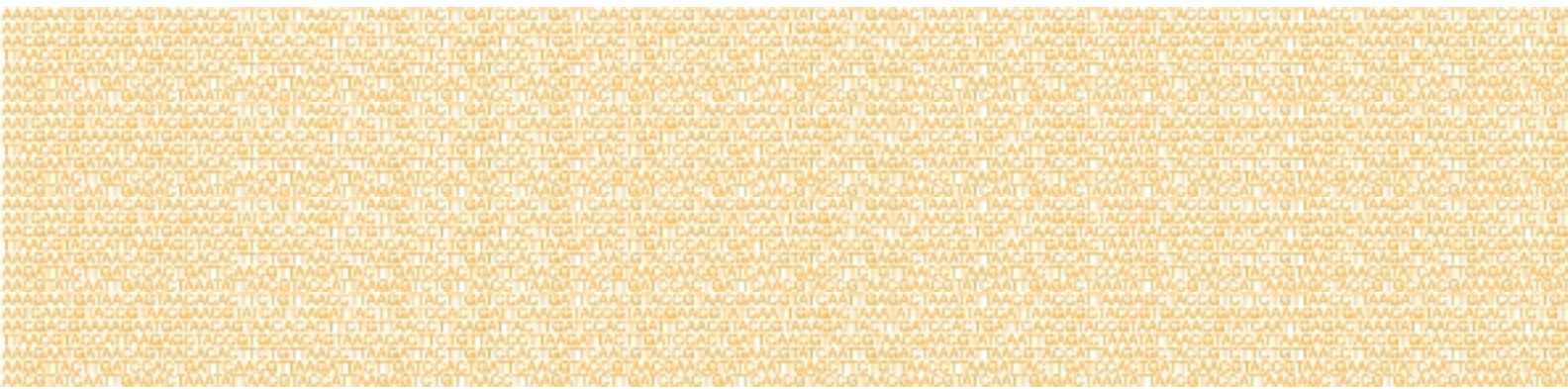
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then click **Documentation & Literature**.



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